Increased Pituitary and Adrenal Reactivity in Premenopausal Women with Posttraumatic Stress Disorder

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Background: *Limited studies of hypothalamic-pituitary-adrenal axis regulation in posttraumatic stress disorder have been performed in premenopausal women. We therefore undertook a study of hypothalamic-pituitary-adrenal axis regulation in this population.*

Methods: *Outpatient posttraumatic stress disorder subjects were compared with healthy, age- and weight-matched nontraumatized subjects. Subjects were free from psychotropic medications, alcohol and other illicit substances for at least 4 weeks before study. Menstrual cycle phase was determined by monitoring the LH surge and plasma progesterone levels. Corticotropin releasing factor and adrenocorticotropin stimulation tests, as well as 24-hour urinary-free cortisol measurements were performed.*

Results: *Corticotropin releasing factor test: Baseline adrenocorticotropic hormone and cortisol levels did not differ between the 12 PTSD and 11 comparison subjects, but the posttraumatic stress disorder group had greater adrenocorticotropic hormone and cortisol responses to corticotropin releasing factor, as well as a later cortisol peak. Adrenocorticotropic hormone test: Baseline cortisol levels did not differ between the 10 posttraumatic stress disorder subjects and seven controls, but the posttraumatic stress disorder group showed greater cortisol responses to adrenocorticotropic hormone. Peak cortisol responses to corticotropin releasing factor and adrenocorticotropic hormone were correlated with each other and with 24-hour urinary-free cortisol excretion.*

Conclusions: *Pituitary and adrenal hyperreactivity to exogenous corticotropin releasing factor and adrenocorticotropic hormone is demonstrated in premenopausal women with chronic posttraumatic stress disorder. Cortisol hyperreactivity thus may play a role in the pathophysiology of posttraumatic stress disorder in women.* Biol Psychiatry 2001;50:965–977

Key Words: PTSD, premenopausal women, stress, ACTH, cortisol, CRF

Introduction

Despite common rates of exposure to traumatic events in males and females, incidence and prevalence rates of posttraumatic stress disorder (PTSD) (APA, 1994) are reported to be approximately twice as high in women as in men (Breslau et al 1995, 1997, 1998; Kessler et al 1995; Stein et al 1997). Nevertheless, most research examining neurobiological factors underlying PTSD have focused on populations of males or postmenopausal females. The exclusion of premenopausal female subjects from these studies could prevent the elucidation of gender- or hormone state-related neurobiologic factors that might confer an increase in the risk for development of PTSD. For instance, the only study examining cortisol output in premenopausal women with PTSD demonstrated high 24-hour urinary-free cortisol levels (Lemieux and Coe 1995). In contrast, some studies in adult males and a study in postmenopausal females have shown baseline cortisol output in PTSD to be low (Mason et al 1986; Yehuda et al 1990; Yehuda et al 1993; Yehuda et al 1995).

High cortisol levels are thought to facilitate the negative effects of stress on the structure and function of the hippocampus (Sapolsky 1985; McEwen et al 1986; Sapolsky 1986; Lupien et al 1998; Newcomer et al 1999), amygdala (Schulkin et al 1998), locus coeruleus (Schulkin et al 1998), and prefrontal cortex (Grundemann et al 1998; Lupien et al 1999), areas of the brain thought to play important roles in the production of symptoms and func-

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Table 1. The Type and Time Course of DSM-IV Criterion A Trauma Experienced by PTSD Subjects

Subject	Age of First Trauma (Years)	Years Between First Trauma and Time of Study	Age of Subsequent Trauma (Years)
1	Physical and emotional abuse (2)[a]	40	Sexual abuse (12–13)
2	Physical and emotional abuse (5)	29	Rape (14)
3	Physical abuse (9)	34	Sexual abuse (13)
4	Witness to parent's sudden death (10)	28	Rape (19), near drowning (20)
5	Rape (12)	9	Physical abuse (14–15)
6	Rape (12)	17	
7	Physical abuse (13)	31	Sexual abuse (14)
8	Abduction and gang rape (16)	26	Witness to suicide (18)
9	Rape in military (22)	6	
10	Domestic violence (33)	1	
11	Gang rape in military (21)	18	
12	Abduction and gang rape (22)	25	
13	Combat (37)	8	

PTSD, posttraumatic stress disorder.
[a]Verified by examination of state records.

tional disabilities associated with chronic PTSD (Sutker et al 1993; Bremner et al 1993; Bremner et al 1995; Gurvits et al 1996; Stein et al 1997; Bremner et al 1997a; Bremner et al 1999; LeDoux et al 1989; Davis et al 1992; Liberzon et al 1999; Morgan et al 1993; McNally et al 1993; Goldstein et al 1996; Bremner et al 1997b; Southwick et al 1999). We thus decided that it would be important to examine hypothalamic-pituitary-adrenal (HPA) axis function more thoroughly in premenopausal women with PTSD to see whether cortisol is high at baseline or in response to pituitary or adrenal activation. In the following study, we used a corticotropin releasing factor (CRF) stimulation test, an adrenocorticotropic hormone (ACTH) stimulation test, and a 24-hour urine collection for free cortisol to examine pituitary and adrenal axis reactivity in a group of premenopausal women with PTSD compared with a group of nontraumatized healthy subjects. Based on several previous reports of decreased urinary-free cortisol levels and increased negative feedback regulation in PTSD and the finding of blunted ACTH responses to CRF in male combat veterans with PTSD (Smith et al 1997; Yehuda 1997), we hypothesized that 24-hour urinary free cortisol levels and ACTH and cortisol responses to CRF and ACTH stimulation would be low in prememopausal women with PTSD compared with nontraumatized healthy controls.

Methods and Materials

Subjects

Subjects with PTSD and healthy nontraumatized comparison subjects (NTC) were recruited via advertisements in community newspapers or referred from the VA Connecticut Healthcare System Anxiety Disorders Clinic to participate in a series of procedures examining HPA axis function approved by the VA Connecticut Healthcare System Human Subjects Subcommittee. Written informed consent was obtained from each subject. Subjects were encouraged, but not required, to participate in all of the endocrine tests described below. All of the procedures were performed at least 1 week apart.

The subjects were screened for current or significant past medical illness, pregnancy, and illicit drug use via medical history, physical examination, and laboratory tests. All were free from psychotropic medications for months to years or never had been treated with psychotropic medications. The most recently treated subject discontinued an antidepressant and a benzodiazepine 4 months before participation. No subjects discontinued psychiatric medications to participate in the study. Subjects abstained from other medications, alcohol, and illicit drugs for at least 4 weeks before testing except for four PTSD subjects taking oral contraceptives.

Trauma exposure was assessed in all subjects using a trauma inventory. Table 1 reports the age of the PTSD subjects at the first and subsequent Criterion A traumas. None of the subjects were currently living in threatening environments or undergoing severe psychosocial adversity.

Healthy NTC subjects were evaluated psychiatrically using the nonpatient edition of the Structured Clinical Interview for DSM-IV (SCID-NP) (First et al 1995a); subjects with a current Axis I diagnosis were excluded. Subjects with PTSD were evaluated using the SCID-P (First et al 1995b) and the Clinician-Administered PTSD Scale (CAPS) lifetime version (Blake et al 1993). Subjects with current or past psychotic disorders, bipolar disorder, or a current eating disorder were excluded.

Among the healthy subjects, one met criteria for a remote past episode of major depression and one for a remote past eating disorder (bulimia). Three subjects were current 1/2 to 1 pack-per-day smokers. Comorbid DSMIV diagnoses among the PTSD subjects included current (2) and past (11) major depression,

current (3) and past (3) dysthymic disorder, current (0) and past (6) substance abuse or dependence (all more than 5 years before participation in the study), current (4) and past (5) simple phobia, current (2) and past (5) agoraphobia without panic disorder, current (0) and past (0) panic disorder, current (2) and past (2) generalized anxiety disorder, current (2) and past (2) obsessive compulsive disorder (symptoms were confined to cleaning rituals triggered by the subjects' rapes), and current (0) and remote past (1) anorexia. Three PTSD subjects were current 1/2 to 1 pack-per-day smokers.

The PTSD and NTC groups as a whole did not differ significantly by age (PTSD: 37.3 ± 2.1 years, NTC: 31.5 ± 2.4 years, $p > .5$) or weight (PTSD: 161.1 ± 9.5 lbs., NTC: 152.3 ± 7.8 lbs., $p > .5$). The subsets of PTSD and NTC subjects participating in each procedure also did not differ by age or weight. All of the PTSD subjects were Caucasian. The NTC subjects were Caucasian (14) and African American (1). Years of education differed significantly but not substantially between groups (PTSD: 13.8 ± .5 vs. NTC: 16.3 ± .5 years, $p < .004$). All subjects had completed high school.

Menstrual Cycle Monitoring

To help determine menstrual cycle phase at the time of each procedure, the subjects used a urinary test kit to detect the midcycle luteinizing hormone (LH) surge (Clear Plan Easy, Whitehall Laboratories, Madison, NJ). Plasma progesterone levels were measured 1 to 2 weeks later to confirm ovulation (Leibenluft et al 1994). Baseline plasma progesterone levels were also measured at the time of each procedure. Progesterone levels were measured by SmithKline Beecham Laboratories by chemiluminescence. For purposes of data analysis, the PTSD subjects on oral contraceptives were considered to be in the follicular phase of the menstrual cycle.

CRF and ACTH Stimulation Tests

The CRF stimulation test was completed by 12 PTSD and 11 NTC subjects. The ACTH stimulation test was completed by 10 PTSD and 7 NTC subjects, all in the follicular phase of the menstrual cycle. The subjects fasted except for water for at least 4 hours and refrained from nicotine for 4 to 6 hours before CRF or ACTH administration. The CAPS-One Week Version (PTSD subjects only), Hamilton Anxiety Scale (Hamilton 1959), and Hamilton Depression Scale-21 Item Version (Hamilton 1967) were administered over a 30-minute period after IV placement 1 hour before injection. Subjects also rated any stressful events that occurred in the previous week using a 14-point Likert scale. Ovine CRF (ACTHREL, Ferring Laboratories) was administered at 8:00 PM at a dose of 1 μg/kg up to a total of 100 μg intravenously over 60 sec. Blood samples were collected at −15, 0, +15, +30, +45, +60, +90, and +120 min after the CRF infusion. $ACTH_{1–24}$ (Cosyntropin, Organon Inc.) was administered at variable times during the day at a dose of 250 μg. The average time of administration did not differ between groups (PTSD: 11:25 AM ± 44 min, range: 9:15 AM to 4:15 PM; NTC: 10:15 AM ± 41 min, range: 8:15 AM to 1:15 PM, $p = .26$). Samples from each time point were spun immediately in a refrigerated centrifuge and stored at −70 degrees C. Plasma ACTH was measured with a coated-bead radiometric sandwich assay using ^{125}I-labeled antibody (Nichols Institute Diagnostics, San Juan, Capistrano, CA). The interassay coefficient of variation (C.V.) was 5.4%. Plasma cortisol was measured using an antibody-coated tube radioimmunoassay (RIA) kit with an interassay C.V. of 8.2% (DiaSorin, Inc., Stillwater, MN).

24-Hour Urine Collections

24-hour urine collections were made by 12 PTSD and 10 NTC subjects in nonacidified containers kept on ice or in a freezer. Urinary free cortisol was measured in the laboratory of John Mason, M.D., and Sheila Wang, Ph.D., at the VA National Center for PTSD, Neuroscience Division, West Haven, using an antibody-coated tube RIA kit (DiaSorin, Inc., Stillwater, MN). Inter- and intra-assay CVs were 6.1% and 3.2%, respectively. Urine collections with creatinine levels exceeding 0.8 mg/24 hours were considered complete.

Data Analyses

For the CRF and ACTH stimulation tests, a random effects model was used to analyze repeated measurements using diagnostic group, time, and menstrual phase (CRF test only) as independent variables. A heterogeneous variance structure, AR(1), was used in the mixed model (Diggle et al 1994). A high-order interaction term was removed from the model if the p value for this term was larger than .1. A t test was used to compare the 24-hour urinary-free cortisol data between groups. A t test for groups with unequal variance was used to compare symptom ratings and other variables between groups when appropriate. Pearson's correlations were used to assess relationships between cortisol responses to ACTH or CRF stimulation, 24-hour urinary-free cortisol levels, and age at the time of initial traumatization. Bonferroni corrections were used to correct for multiple comparisons. Significance was otherwise set at $p < .05$ and results were considered marginally significant if $.05 \leq p < .10$. Group means are expressed ± the standard error of the mean (SEM). "Baseline" plasma levels are expressed as the mean of the −15′ and +0′ time points relative to the CRF or ACTH injections. Missing values were imputed by carrying the last observation forward, except for two values occurring immediately after CRF or ACTH injection. These were imputed as the average of the baseline and following time points.

Results

CRF Stimulation Test: Effects on Plasma ACTH and Cortisol Levels

Plasma ACTH levels increased after CRF in both the PTSD and NTC groups: $F(6, 126) = 15.12$, $p < .0001$, but there was a trend for a larger ACTH response to CRF in the PTSD group as indicated by a group by time effect:

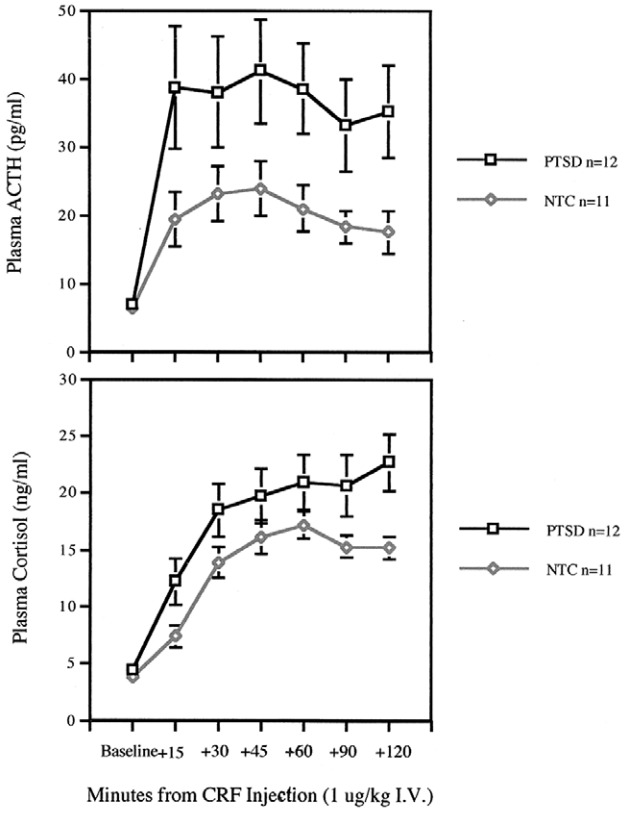

Figure 1. Plasma adrenocorticotropic hormone (ACTH) (a) and plasma cortisol (b) responses to the intravenous (I.V.) administration of ovine corticotropin releasing factor (ovine CRF: ACTHREL, 1 μg/kg) in premenopausal women with PTSD and nontraumatized comparison (NTC) subjects. As presented in the results section, the PTSD group showed a trend for an increased ACTH response across time and a significantly increased peak change in ACTH after CRF. The PTSD group also showed a significantly increased cortisol response to CRF compared to the NTC subjects and a later peak in plasma cortisol after CRF. Values at each time point are expressed as the mean ± SEM.

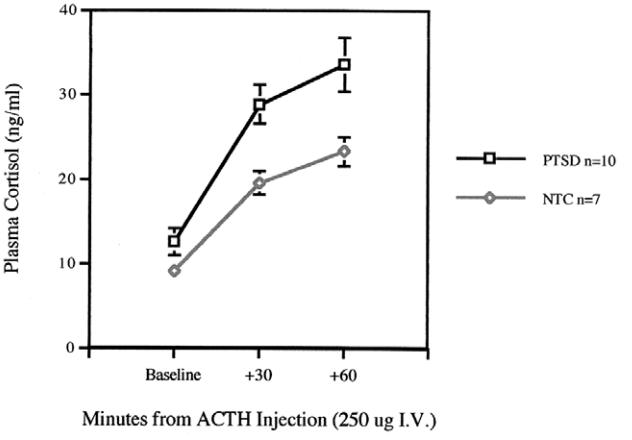

Figure 2. Plasma cortisol responses to the intravenous (I.V.) injection of a maximally stimulating dose of adrenocorticotropin (ACTH: Cosyntropin, 250 μg) in premenopausal women with PTSD and nontraumatized comparison (NTC) subjects. As presented in the results section, plasma cortisol increased more in the PTSD group compared to the NTC group. All subjects were in the follicular phase of the menstrual cycle. Values at each time point are expressed as the mean ± SEM.

$F(6126) = 2.10$, $p = .058$. (Figure 1a). Indeed, while the mean plasma ACTH level did not differ between groups at baseline: (PTSD: 7.0 ± 1.6 vs. NTC: 7.0 ± 2.3 pg/mL, $t(21) = -.31$, $p = .76$.), the peak change in plasma ACTH was greater in the PTSD group (PTSD: 40.3 ± 26.0 vs. NTC: 21.6 ± 11.4 pg/mL, $p < .04$). The group by time by phase interaction was not significant: $F(6114) = .58$, $p = .75$, and there was no effect of menstrual phase on plasma ACTH levels: $F(1,19) = .13$, $p = .72$. In addition, baseline progesterone levels did not differ between groups (PTSD: 3.8 ± 2.1, NTC: 6.2 ± 2.2 ng/mL, $p = .45$). Finally, when the four PTSD subjects on oral contraceptives were removed from the analysis, the results were essentially unchanged. The ACTH response to CRF across time remained marginally larger in the PTSD group: $F(6, 102) = 2.89$, $p < .10$, and the peak change in ACTH in response to CRF was significantly greater in the PTSD group ($p < .05$).

Plasma cortisol levels also increased after CRF in both groups: $F(6, 126) = 50.81$, $p < .0001$, but there was a larger cortisol response in the PTSD subjects as indicated by a significant group by time effect: $F(6126) = 2.66$, $p < .05$ (see Figure 1b). Indeed, while baseline cortisol levels did not differ between groups (PTSD: 4.38 ± .70 vs. NTC: 3.84 ± 1.02 ng/mL,: $t(21) = -.53$, $p = .60$), there was a greater peak change in plasma cortisol in the PTSD group (PTSD: 19.1 ± 2.00 vs. NTC: 14.1 ± 1.2 ng/mL, $p < .05$). In addition, the cortisol peak after CRF was later in the PTSD group (PTSD: 97 ± 7 vs. NTC: 68 ± 9 min, $p < .02$). The group by time by phase interaction was not significant: $F(6, 114) = .96$, $p = .46$, and there was no effect of menstrual phase on cortisol responses after CRF: $F(1,20) = 1.55$, $p = .23$. In addition, when the four PTSD subjects on oral contraceptives were removed from analysis, the cortisol response to CRF remained significantly greater in the PTSD group: $F(6, 102) = 2.89$, $p < .05$.

Among the PTSD subjects, there was a strong negative correlation between age of first trauma and the peak change in cortisol after CRF ($r = -.74$, $p = .004$). There was a positive correlation between the years since first trauma and the peak change in cortisol ($r = .61$, $p = .03$). The age of the PTSD subjects at the time of this study did not correlate with peak cortisol responses to CRF stimulation and ACTH responses did not correlate with age of first trauma.

ACTH Stimulation Test: Effects on Plasma Cortisol Levels

Plasma cortisol increased after ACTH in both groups: $F(2,30) = 130.45$, $p < .0001$, but increased more so in the PTSD group: $F(2, 30) = 6.02$, $p < .01$ (Figure 2). There also was a trend for an increase in baseline plasma cortisol in the PTSD group (PTSD: 12.6 ± .9 vs. NTC: 9.1 ± 1.6 ng/mL); however, when the three PTSD subjects on oral contraceptives were removed from analysis, there was no longer a significant difference in baseline cortisol levels (PTSD: 11.5 ± 1.4, NTC: 9.1 ± 1.6 ng/mL), but the cortisol response to ACTH remained greater in the PTSD group: $F(2, 24) = 5.07$, $p < .05$). Baseline progesterone levels did not differ between groups (PTSD: .44 ± .08 vs. .45+ .05 ng/mL, $p = .97$).

Stress and Symptom Ratings for the Week Preceding the CRF and ACTH Tests

The number of stressful events reported during the week preceding the CRF and ACTH tests did not differ between groups (CRF test: PTSD-1.2 ± .4 vs. NTC- .7 ± .3; ACTH test: PTSD- .7 ± .3 vs. NTC- .3 + .2); however, the intensity of the most stressful event was greater in the PTSD group (CRF test: PTSD-10.9 ± 1.4 vs. NTC-6.0 ± 1.3, $p < .04$; ACTH test: PTSD-12.1 ± 1.1 vs. NTC-5.0 ± 1.0, $p < .02$).

As expected, Ham-A scores were higher in the PTSD compared with NTC subjects (CRF test: PTSD-14.4 ± 2.5 vs. NTC- .6 ± .4; ACTH test: PTSD-13.2 ± 2.2 vs. NTC-1.4 ± .4; $p < .0001$). Ham-D scores were also higher in the PTSD subjects (CRF test: PTSD-12.1 ± 2.2 vs. NTC-1.3 ± .6; ACTH test: PTSD-10.9 ± 1.7 vs. NTC-0.7 ± .3, $p < .0001$). The mean total CAPS score was 42.7 ± 7.4, range: 18–84 for the CRF test and 43.5 ± 7.5, range: 11–92 for the ACTH test. Total CAPS scores correlated with Ham-A scores (CRF test: $r = .76$, $p < .01$; ACTH test: $r = .65$, $p < .05$) and Ham-D scores (CRF test: $r = .74$, $p < .01$; ACTH test: $r = .63$, $p < .05$). Ham-A and Ham-D scores were also highly correlated (CRF test: $r = .96$; ACTH test: $r = .94$, $p < .0001$). There were no significant relationships between cortisol responses to CRF or ACTH and symptom ratings in the PTSD group.

24-Hour Urinary-free Cortisol Levels

Urine collections were complete in 12 PTSD and 8 NTC subjects. There was no effect of menstrual phase on urinary-free cortisol levels. Menstrual phase was thus removed from the analysis. A one-way ANOVA revealed no significant difference between groups in total 24-hour urinary cortisol (PTSD: 42.8 ± 4.2 vs. NTC: 34.6 ± 4.8 ng/day, $p = .22$); however, there was a trend for a 29% increase in the 24-hour urinary cortisol/creatinine ratio in the PTSD group (PTSD: 38.3 ± 3.1 vs. NTC: 29.8 ± 3.5 ng/mg, $p < .10$). There was no difference between groups in the output of creatinine per day (PTSD: 1.1 ± .06 vs. NTC: 1.1 ± .05 ng/day, $p = .75$).

Correlations Among Cortisol Responses During the Different Procedures

Ten PTSD subjects and seven NTC subjects completed both the CRF and ACTH stimulation tests. There were positive correlations between peak cortisol levels after CRF and ACTH ($r = .73$, $p < .001$) and between peak changes in cortisol in response to CRF and ACTH ($r = .62$, $p < .01$).

Eleven PTSD and seven NTC subjects completed both the 24-hour urine collection and the CRF stimulation test. There were positive correlations between 24-hour urinary cortisol levels and CRF-stimulated peak cortisol levels ($r = .58$, $p < .02$) and between 24-hour urinary cortisol levels and peak cortisol responses to CRF ($r = .56$, $p < .02$).

Ten PTSD subjects and three NTC subjects completed both the 24-hour urine collection and the ACTH stimulation test. There were positive correlations between 24-hour urinary cortisol levels and ACTH stimulated peak cortisol levels ($r = .68$, $p < .01$) and between 24-hour urinary cortisol levels and peak cortisol responses to ACTH ($r = .70$, $p < .01$).

Discussion

In this study, we found increased ACTH and cortisol responses to ovine CRF administration, and increased cortisol responses to ACTH administration in a group of premenopausal women with chronic PTSD compared with healthy nontraumatized comparison subjects. In addition, there were significant positive correlations between cortisol responses to CRF stimulation, cortisol responses to ACTH stimulation, and 24-hour urinary-free cortisol levels. The current study thus raises questions about previous assertions that "low baseline cortisol levels and increased negative feedback regulation" (Yehuda et al 1997) and "hypocortisolism" (Heim et al 2000) characterize PTSD. The data also suggest that increased cortisol reactivity may play a role in the pathophysiology of PTSD in premenopausal women and could contribute to structural and functional changes in the brains of affected patients.

Comparison to Results of Previous HPA Axis Studies in PTSD

CRF AND ACTH STUDIES. The findings of the current study are consistent with the work of Kaufman et al (1997)

showing a greater ACTH response to CRF among 13 depressed abused children (8 with PTSD) compared with 13 healthy controls and 13 depressed nonabused children (0 with PTSD). When the depressed abused group was divided into high versus low ACTH responders, there was a trend for a greater number of subjects with PTSD to be among the high responders. The current work is also consistent with recent work by Heim et al (2000) showing increased ACTH and cortisol responses to a laboratory psychosocial stress test in 13 premenopausal women with major depression and childhood trauma (11 with PTSD) compared with 14 women with childhood trauma, but no depression (5 with PTSD). Two other studies also suggest the presence of increased pituitary and adrenal reactivity in PTSD. A 24-hour plasma sampling study by Yehuda et al (1996a) showed male Vietnam veterans to have greater cortisol diurnal variation and greater signal to noise ratios compared with healthy controls and patients with major depression. Yehuda et al (1996b) also found increased ACTH release after metyrapone-induced inhibition of cortisol production in veterans with PTSD. This has been interpreted to suggest the presence of enhanced glucocorticoid negative feedback in PTSD, but the current study suggests that greater pituitary sensitivity to CRF also may have contributed to the findings.

In contrast, the results of the current CRF study in women (see Figure 1) differ from those of Smith et al (1989) and Heim et al (2001). In the Smith study, eight male veterans with PTSD showed blunted ACTH and normal cortisol responses to CRF compared with a healthy group of four combat controls and seven nontraumatized subjects. Of the eight subjects with PTSD, four had concurrent major depression, a diagnosis previously associated with blunted ACTH responses to CRF (De Bellis et al 1994; Gold et al 1995; Arborelius et al 1999). In the Heim study, women with childhood abuse and major depression (19/20 with PTSD) showed lower absolute ACTH levels at 60 and 120 min after CRF and lower cortisol levels at 0, 5, 90, and 120 min after CRF compared with healthy women without childhood abuse. The women with childhood abuse and depression showed lower baseline cortisol levels during an ACTH stimulation test.

The presence of comorbid major depression therefore could account for blunted ACTH responses in PTSD; however, as previously noted, a blunted ACTH response to CRF was not found in depressed children with PTSD (Kaufman et al 1995). In addition, the ACTH and cortisol levels and responses of the two PTSD subjects with major depression in the current study were within one-half of a SD of the PTSD group means. Thus factors other than depression may contribute to the variable results among CRF and ACTH studies in PTSD.

For instance, antidepressants, neuroleptics, and anxiolytics can suppress HPA axis activity (Tandon et al 1991; Brady et al 1992; Vargas et al 1992; Fuchs et al 1996; Gold et al 1995; Barden 1996; Steptoe et al 1996; Fuchs et al 1997; Rowe et al 1997; Thakore 1997). In the study by Smith et al (1989), psychotropic medications were discontinued in the PTSD subjects only 7 days before testing. The elapsed time since discontinuation of psychotropic medications was not reported by Heim et al (2001). Subjects in the current study and in the study by Kaufman et al (1995) had not been treated with psychotropic medications for extended periods, if at all.

Nicotine also has effects on HPA axis function. While acute nicotine administration increases plasma cortisol levels (Spohr et al 1980; Sellini et al 1989; Coiro et al 1999), *chronic nicotine use* has been associated with increased baseline plasma and urinary cortisol levels (Eliasson et al 1993; Baron et al 1995) and with decreased baseline and stimulated plasma cortisol levels (Sellini 1989; Kirschbaum et al 1994; Krishnan-Sarin et al 1999). In addition, recent smoking, compared with overnight abstinence, suppresses cortisol reactivity to mental stress (Tsuda et al 1996). In the current study, nicotine use was matched between groups. In the study by Kaufman et al (1995), there were no smokers. Smoking status was not controlled by Smith et al (1985) or Heim et al (2001), even though there is typically a much higher prevalence and intensity of smoking in PTSD (Shalev et al 1990; Beckham et al 1997).

Differences in alcohol consumption among subjects also may have contributed to differences in results among studies. Acute alcohol administration increases cortisol levels during the subsequent period of mild withdrawal (Ekman et al 1994; Sarkola et al 1999), while moderate chronic use suppresses cortisol reactivity with effects persisting in alcohol dependent subjects up to 4 weeks after the establishment of abstinence (Costa et al 1996; Bernardy et al 1996). While subjects with current alcohol abuse or dependence were excluded from all CRF and ACTH studies in PTSD to date, the nonabusive use of alcohol was allowed but not quantified by Heim et al (2001) and the length of alcohol abstinence may have been as little as 7 days in the study by Smith et al (1989).

Finally, the distribution of oral contraceptive users across the healthy and depressed abused groups was not reported by Heim et al (2001). Oral contraceptives increase plasma cortisol binding globulin levels and thereby may increase cortisol levels without altering reactivity (Kirschbaum et al 1999).

24-HOUR URINARY CORTISOL STUDIES. The finding of a marginally significant increase in urinary-free cortisol in premenopausal women with PTSD in the current study also contrasts with some, but not most previous studies in PTSD. In particular, this result is consistent with a study in

premenopausal women showing a 27% increase in urinary-free cortisol in women with PTSD due to early childhood sexual abuse compared with nontraumatized controls (Lemieux and Coe 1995).

When comparing the results of 24-hour urinary-free cortisol studies, it is again important to consider differences in experimental design. For instance, some earlier studies included PTSD subjects taking or only recently discontinued from a variety of psychotropic medications (Mason et al 1986; Yehuda et al 1990, 1993; Kosten et al 1990; Lemieux and Coe 1995). Others did not require prolonged abstinence from alcohol (Mason et al 1986; Yehuda et al 1990, 1993, 1995; Kosten et al 1990; Maes et al 1998) and none controlled for nicotine use.

In addition, it should be remembered that 24-hour urinary cortisol measurements reflect both baseline and reactive changes in plasma-free cortisol levels across time. In studies showing urinary-free cortisol levels in PTSD subjects to be low or not different than controls (Mason et al 1986; Yehuda et al 1990, 1993, 1995; Kosten et al 1990; Baker et al 1999), the PTSD subjects were *confined* to home or hospital environments. These subjects may have experienced fewer environmental provocations or less novelty than PTSD subjects participating in studies that allowed free access to the community, all of which have shown increased urinary-free cortisol levels in PTSD (Pitman and Orr 1990; Lemieux and Coe 1995; De Bellis et al 1999; Maes et al 1999, the current study). This suggestion is supported by the work of Aronsson and Rissler (1998), showing reduced cortisol levels on days off work compared with days at work even among healthy subjects. Alternatively, it is possible that urinary cortisol levels are lower in more symptomatic PTSD subjects, as suggested by Yehuda et al (1995) and Baker et al (1999). Studies of hospitalized PTSD patients would tend to contain such subjects; however, even among studies of hospitalized PTSD patients (Mason et al 1986; Yehuda et al 1990, 1993; Kosten et al 1990; Baker et al 1999; Mason et al 2001), the extent to which patients are allowed to utilize avoidant coping defenses may affect urinary cortisol levels (Mason et al 2001). In addition, low sodium diets sometimes used to treat hypertension can reduce urinary-free cortisol (Lewicka et al 1998).

Factors of Possible Relevance to Upregulation of the HPA Axis in PTSD

The pattern of HPA axis reactivity seen in the premenopausal women with PTSD in the current study has been seen previously under a variety of conditions. This may provide clues to mechanisms underlying upregulation of the HPA axis in these subjects.

For instance, maternal separation stress in neonatal rats induces increased HPA axis responding to stress in adult animals (Francis et al 2000). This may be relevant to the observed negative correlation between age of first trauma and cortisol reactivity in the current study; however, the subjects with childhood trauma also reported more traumas (see Table 1), consistent with studies linking the risk for PTSD to overall trauma exposure (Breslau et al 1995; Hubbard et al 1995; Schaaf and McCanne 1998) and with studies showing an increased risk for subsequent trauma in persons with childhood abuse (Farley and Barkan 1998; Mueser et al 1998). Alternatively, the correlation between time elapsed since first trauma and cortisol reactivity may reflect progressive detrimental effects of cortisol on the hippocampus and secondary disinhibition of the HPA axis (Lupien et al 1998; Jacobson and Sapolsky 1991; De Kloet et al 1998).

An enhancement of pituitary and adrenal reactivity in PTSD also could be mediated by vasopressin-potentiation of ACTH responses to novelty (Dallman et al 1973; Aguilera 1994; Marti et al 1994; Aguilera 1998), as well as an increase in the central perception of novelty among subjects with PTSD (Morgan and Grillon 1999; Kimble et al 2000).

Genetic factors also may play a role. Witchel et al (1997) found increased cortisol responses to ACTH, while Merke et al (2000) found greater ACTH and cortisol responses to CRF in persons with heterozygosity for common mutations of the adrenal 21-hydroxylase gene. Indeed, the high rate of these mutations among certain ethnic populations may have contributed to the variable results across HPA axis function studies in PTSD.

HPA axis upregulation also has been observed in animals and humans treated with RU486 (Van Haarst et al 1996; Bertagna et al 1994). This has been attributed to the antiglucocorticoid effects of RU486 and raises the possibility that endogenous steroids with antiglucocorticoid activity, such as DHEA or progesterone, may play a role in altering HPA axis activity in PTSD (Chader and Reif-Leher 1972; Kaiser et al 1979; Bohus and DeKloet 1981; Giesen and Beck 1982; Chou and Luttge 1988; Daynes et al 1990; Blauer et al 1991; Browne et al 1992; Araneo et al 1993).

Finally, female gender or premenopausal status within female gender may exert unique influences on the pattern of HPA axis adaptation to trauma. Both estrogen (Lindholm and Schultz-Moller 1973; Norman et al 1992; Vamvakopoulos and Chrousos 1993; Handa et al 1994; Lesniewska et al 1996; Kirschbaum et al 1996; Chrousos et al 1998; De Leo et al 1998; Komesaroff et al 1999) and testosterone (Handa et al 1993; Handa et al 1994; Suescun 1994; Viau et al 1999) have effects on HPA axis reactivity

and may play a role in gender specific adaptations to trauma.

Study Limitations

The relatively small number of subjects participating in the current study limits the confidence with which conclusions can be drawn from the results. The lack of a trauma-exposed group without PTSD also prevents attribution of the findings to PTSD rather than trauma. There are also specific experimental design flaws that may have influenced the results. For instance, baseline progesterone levels were generally controlled between groups by monitoring menstrual phase, but estrogen status was not and may have influenced cortisol responses to ACTH (Kruyt and Rolland 1982). It is also possible that stress associated with the administration of the CAPS during the hour before CRF or ACTH administration influenced the PTSD group responses. The PTSD subjects did have higher baseline heart rates (CRF test: PTSD-72 $\pm$ 4 bpm [beats per minute] vs. NTC-58 $\pm$ 3 bpm, $p < .05$; ACTH test: PTSD-74 $\pm$ 3 bpm vs. 65 $\pm$ 2 bpm, $p < .02$), suggesting the presence of increased sympathetic tone; however, an increase in "baseline" heart rate is typically seen in PTSD subjects participating in laboratory challenges and has been attributed to increased anticipatory anxiety (Prins et al 1995). Indeed, other neurobiologic factors associated with PTSD such as noradrenergic hyperreactivity (Southwick et al 1999) and alterations in NPY physiology (Rasmusson et al 2000) may affect sympathetic tone as well as HPA axis reactivity (Suda et al 1993; Pralong et al 1993). In addition, it seems unlikely that a state of greater psychological stress would account for the increased *ACTH-induced cortisol responses* in the PTSD group since this is a test of adrenal capacity. Also, psychological stress is thought to increases baseline cortisol levels so that exogenous ACTH may not be able to induce as much of a cortisol response (Orth and Kovacs 1998). The difference in timing of ACTH administration between groups, though not significant, is also not methodologically optimum (Orth and Kovacs 1998); however, this is again unlikely to account for the difference in cortisol responses to ACTH. According to Dickstein et al (1991), *immediate* cortisol responses to ACTH are greater when ACTH is administered at 4:00 PM compared with 8:00 AM, but this difference is abolished by 30 min after ACTH administration. In addition, if we eliminate the PTSD subject studied at 4:15 PM and the NTC subject studied at 8:25 AM so that the mean time of ACTH administration is clearly not different between groups (PTSD: 10:53 AM $\pm$ 34 min, range: 9:25 AM –1:45 PM vs. NTC: 10:35 AM $\pm$ 42 min, range: 9:15 AM to 1:15 PM, $p = .73$), the finding of an increased cortisol response to ACTH in the PTSD group remains significant.

Summary and Future Directions

This study has demonstrated increased pituitary and adrenal reactivity to exogenous CRF and ACTH that correlates with 24-hour urinary-free cortisol excretion in premenopausal women with chronic PTSD. These findings suggest that conclusions regarding low cortisol output in PTSD are premature and raise the possibility that risk to brain structure and function in PTSD may be mediated, in part, by reactive increases in cortisol; however, further studies must determine whether these findings can be replicated in premenopausal women and other populations of PTSD patients. It also will be important to study healthy subjects with histories of trauma. In addition, "because cortisol levels are affected by environmental conditions, particularly acute stress, exercise, smoking, and so forth, it is essential to standardize the experimental conditions under which samples are obtained, and include descriptions of these conditions in the methods sections of scientific reports (Yehuda 1999)." Hopefully, future efforts along these lines will lead to a better understanding of the pathophysiology of PTSD, identification of risk factors for PTSD and comorbid conditions such as major depression, and better approaches to the prevention and treatment of this prevalent and disabling neuropsychiatric disorder.

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